IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Examiner: J. Brusca
COX III et al.	Group Art Unit: 1631
For: **REGULATION OF ENDOGENOUS GENE EXPRESSION IN CELLS USING ZINC FINGER PROTEINS**	Confirmation No.: 6940
Serial No.: 09/706,243	**DECLARATION PURSUANT TO 37 C.F.R. § 1.132 OF CASEY C. CASEY, PhD.**
Filed: November 3, 2000	
Atty. Docket No.: 8325-0002.10 (S2-US3)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

Dear Sir:

I, Casey C. Case, hereby declare as follows:

1. I am currently Vice President, Research Operations at Sangamo BioSciences. I have held senior research management positions at Sangamo since 1997. Prior to joining Sangamo, I was Director of Cell Biology at Tularik, a prominent Bay Area biotechnology company. Prior to that I was Director of Transcription Research at Oncogene Science (now called OSI Pharmaceuticals). I received my Ph.D. in Biochemistry from the University of California, Davis and postdoctoral training at UCLA. A copy of my Curriculum Vitae (Exhibit A) is attached hereto.

2. I am extremely familiar with *in vivo* and *in vitro* studies of involving engineered zinc finger proteins. I have co-authored numerous publications and patents in the field of gene expression, including publications relating to zinc finger proteins.

3. I have reviewed the following documents: (1) relevant portions of pending Patent Application Serial No. 09/706,243 for "REGULATION OF ENDOGENOUS GENE EXPRESSION USING ZINC FINGER PROTEINS" by Cox et al., of which I am a co-inventor, (hereinafter "the specification"); (2) the Declaration by Dr. Pabo; and (3) Yeh et al. (2003) Abstract #1191 *J. Am. Soc. Gene Therapy* 7(5) May2003:S461).

4. As the data depicted in Figures 1-6 of Exhibit B shows, experiments have been conducted demonstrating that engineered zinc finger proteins (ZFPs), delivered in protein form,

modulate endogenous VEGF gene expression *in vitro* and *in vivo*. These experiments were summarized in Yeh, which was previously made of record in this case. We at Sangamo BioSciences provided polynucleotides encoding engineered ZFPs targeted to VEGF genes used for these experiments. As shown in Figure 1 of Exhibit B, standard site-directed insertional mutagenesis techniques were used to subclone a variety of internalization peptide sequences (IS) into the 5' end of Sangamo constructs. As shown in Figure 2, a functional domain (Activation) was subcloned onto the 3' end of the Sangamo constructs. Thus, the exemplary construct shown in Figure 2 encodes a fusion protein comprising a histidine tag sequence (HIS), an internalization peptide sequence (EP), a nuclear localization signal (NLS), a VEGF-ZFP (ZFP DNA binding) and a functional domain (Activation). Also shown in Figure 2 is the experimental protocol for making these constructs and expressing the resulting fusion proteins. The protocols correspond essentially to the techniques set forth on pages 33-36 of the specification. Yeh et al. used various peptide internalization sequences (IS), including sequences selected by phage display (EP or PPD) and antennapedia (AP), as described on page 44 of the specification. ZFPs and ZFP-IS fusion proteins were delivered *in vitro* or *in vivo* by standard techniques, for example as described in the last paragraph of page 49 of the specification. The effect of the ZFPs on VEGF-A mRNA levels was measured after time, using mRNA detection assays, essentially as described on page 37, lines 14-21 of the specification.

5. Figures 3-6 of Exhibit B show that VEGF expression was enhanced *in vivo* and *in vitro* upon a single application of ZFP-IS fusion proteins. In particular, Figure 3 of Exhibit B shows the results of an *in vitro* experiment, and demonstrates that an internalization sequence mediates transport of ZFPs into cells. Figure 4 shows the large percent increase in VEGFA mRNA expression *in vitro* after administration of VEGF-targeted ZFP fusion proteins. Figure 5 is a graph depicting transduction efficiency of a VEGF-ZFP-IS fusion protein, in which the internalization sequence used is either a phage display selected peptide (EP) or antennapedia (AP). Figure 5 demonstrates that both EP and AP internalization sequences significantly increase the levels of VEGF mRNA in cells. Figure 6 of Exhibit B shows *in vivo* activation of VEGF-A by two different ZFP-IS fusion proteins (IP1-VEGF ZFP and IP2-VEGF ZFP) after injection into a mouse hindlimb skeletal muscle. The levels of VEGFA mRNA increased at least 200% *in vivo* after administration of ZFP-IS proteins. Thus, using the VEGF-targeted ZFPs provided by Sangamo Biosciences, it was demonstrated that direct fusion protein transduction is an effective way to upregulate VEGF-A transcription *in vitro* and *in vivo*.

6. These results demonstrate that engineered ZFPs can be used to modulate expression of endogenous genes *in vitro* and in living animals when delivered as proteins. In addition, the data demonstrates that a variety of protein delivery mediators (*e.g.*, PPDs, antennapedia, and the like) can be used. Furthermore, because ZFPs can be designed and/or

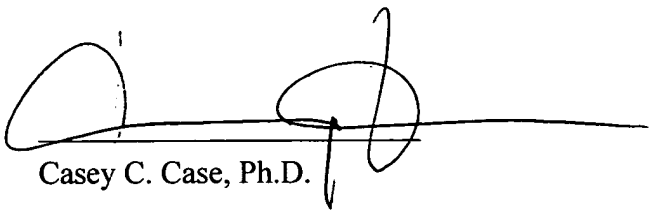
selected to bind to any predetermined sequence, methods similar to those described in this declaration with regard to delivery of VEGFA-targeted ZFPs in protein form are equally applicable to any endogenous gene of interest.

7. Therefore, I agree with Dr. Pabo's conclusion as set forth in his Declaration that, as a technical matter, a skilled worker could have readily delivered ZFPs in protein form to cells in view of the teachings of the specification, together with the common general knowledge, tools and methods available as of the effective filing date of January 1999.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

10-16-03

Date



Casey C. Case, Ph.D.

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October 8, 2003

Core Expertise/Summary: Builder of scientific teams and operations; Communicator of scientific accomplishments and vision; Solid reputation for using cell and animal-based approaches for the discovery of novel therapeutics; Expert in the regulation of gene expression.

Professional Experience

Sangamo BioSciences, Inc. **Point Richmond, California**
A biotechnology company using engineered zinc finger-based transcription factors for the control of gene expression. The technology is being used to study gene function and to develop new therapies.

Vice President, Research Operations **2003-Present**
Vice President, Research **1997-2003**

- Built and led an excellent scientific staff from zero to 86 scientists (35 with Ph.D.s).
- Established partnerships with over 20 pharmaceutical and large biotech companies.
- Proved the utility of Sangamo's technology for pharmaceutical target validation, mouse models, transgenic plants and gene therapy.
- Along with the CEO, pitched the Sangamo vision to potential investors during the IPO road show. We raised $60 million.
- Authored multiple successful grant applications, patent applications, book chapters and peer-reviewed articles.
- Led a cardiovascular gene therapy effort from concept to bench to pre-IND.

Tularik, Inc. **South San Francisco, California**
A bio-pharmaceutical company discovering and developing drugs that act at the level of gene expression.

Director, Cell Biology **1994-1997**
Director, Assay Development **1993-1994**

- Established and led a department using cell-based methods to discover and develop drug candidates (this grew to a group of 20 scientists, 10 with Ph.D.s).
- Developed assays and automation able to screen over 100,000 compounds against multiple targets in six months.
- Provided cell biology data and input for two successful oncology INDs.
- Was the scientific leader for the design and construction of 60,000 square feet of laboratory space.
- Established a tissue culture core facility to provide the company with high quality cells (both established cell lines and primary cells) for experimental purposes.

Oncogene Science, Inc. **Uni ndale, New York**
Now called OSI Pharmaceuticals, Oncogene Science is a biopharmaceutical company discovering and developing drugs, principally focusing on cancer.

Director, Transcription Research	**1992-1993**
Associate Director, Transcription Research	**1991-1992**
Program Manager, Special Projects	**1990-1991**
Senior Research Scientist, Molecular Genetics	**1989-1990**

- Developed a method using engineered cell lines, laboratory robots and reporter genes to discover drugs that act at the level of gene expression.
- Managed several collaborations with large pharmaceutical companies.
- Established a separate department (Special Projects) entirely funded by federal grants (raised over $2 million).
- Drafted claims and supporting documentation for issued U.S. patents.
- Member of the senior management committee.

Postdoctoral Fellow **1985-1989**
UCLA. Robert Simons' laboratory. Department of Microbiology and Molecular Genetics

- Molecular mechanisms controlling the rate of Tn10 transposition.
- Discovered molecular mechanism of inhibition of the transposase gene by a naturally occurring antisense RNA.
- Characterized the half-life of the mRNA, the antisense RNA and many mutants.
- Established RNA methods still in use in several of the department's labs.
- Analysis of promoter mutants.

Ph.D., Biochemistry **1980-1985**
UC Davis. Merna Villarejo's laboratory. Department of Biochemistry and Biophysics.

- The control of gene expression by osmolarity.
- Discovered cross talk between osmolarity regulation and phosphate regulation.
- Discovered connection between osmolarity regulation and gene expression effects caused by local anesthetics.

Education

Ph.D., Biochemistry. 1985. University of California, Davis.
B.S., Biology. 1978. San Diego State University.

Memberships

American Society for Biochemistry and Molecular Biology
Society for Biomolecular Screening
Association for Laboratory Automation
American Society for Gene Therapy

Grants and Awards

As Author and/or Principle Investigator

1983	Jastro-Shields Predoctoral Award
1985	California Institute for Cancer Research, Postdoctoral Fellowship
1986	American Cancer Society, Postdoctoral Fellowship
1990	New York State Science and Technology Foundation Grant "The Discovery and Development of Novel Anti-Fungal Drugs"
1991	NIH NHLBI SBIR "Transcriptional Inhibition of the Scavenger Receptor"
1991	NIH NIDKKD SBIR "Transcriptional Control of the Erythropoietin Gene"
1992	NIH NHLBI SBIR "Inhibition of MCP-1 Gene Expression and Atherogenesis"
1992	NIH NHLBI SBIR Phase II "Transcriptional Inhibition of the Scavenger Receptor"
1992	NIH NCI SBIR "Seeking Drugs that Increase Kit Ligand Expression"
1993	NIH NIDDK SBIR Phase II "Development of Growth Hormone Releasing Factor Agonists"
1993	NIH NHLBI SBIR Phase II "Inhibition of MCP-1 Gene Expression and Atherogenesis"
1998	NIST Advanced Technology Program "Development of Novel DNA Binding Proteins as Antiviral Therapeutics"

As Lab Head and Director:

1997	USDA SBIR "Development of Novel DNA Binding Proteins for Gene Regulation in Soybean"
1998	NIH NHLBI SBIR "Therapeutic Angiogenesis: Novel VEGF Activation Mechanism"
1998	NIH NIAID SBIR "Targeted CpG Methylation of CCR5 Gene: Novel HIV Therapy"

Grants and Awards (Cont.)

1999	NIH NCI SBIR "Engineered Transcription Repressors as Tool for Analyzing Cancer Genes"
1999	NIH NCI SBIR "Inhibiting Heparinase Transcription for Cancer Therapy"
2002	NIH NCI SBIR Phase II "Targeted VEGF Methylation: Novel Cancer Therapy"

Issued Patents

US 5,580,722
"Methods of Determining Chemicals that Modulate Transcriptionally Expression of Genes Associated with Cardiovascular Disease"

US 5,846,720
"Methods of Determining Chemicals that modulate Expression of Genes Associated with Cardiovascular Disease"

US 6,165,712
"Method of Transcriptionally Modulating Expression of Viral Genes and Genes Useful for Production of Proteins"

US 6,453,242
"Selection of Sites for Targeting by Zinc Finger Proteins and Methods of Designing Zinc Finger Proteins to Bind to Pre-selected Sites"

US 6,503,717
"Methods of Using Randomized Libraries of Zinc Finger Proteins for the Identification of Gene Function"

US 6,534,261
"Regulation of Endogenous Gene Expression in Cells Using Zinc Finger Proteins"

US 6,599,692
"Functional Genomics Using Zinc Finger Proteins"

US 6,610,489
"Pharmacogenomics and Identification of Drug Targets by Reconstruction of Signal Transduction Pathways Based on Sequences of Accessible Regions"

GB 2,348,424
"Use of Zinc Finger Proteins to Regulate Gene Expression"

GB 2,348,425
"Selection of Sites for Targeting by Zinc Finger Proteins and Methods of Designing Zinc Finger Proteins to Bind to Pre-selected Sites"

GB 2,360,285
"Design and Synthesis of Zinc Finger Proteins"

Publicati ns

Van Eenennaam, A.L., Li, G., Venkatramesh, M., Levering, C., Gong, X., Jamieson, A.C., Shewmaker, C.K. and **Case, C.C.** (2003) Elevation of Seed α-tocopherol Levels using Plant-based Transcription Factors to an Endogenous Locus. Metabolic Engineering. In Press.

Lui, P-Q., Morton, M.F., Reik, A., de la Rosa, R., Mendel, M.C., Li, X-Y., **Case, C.C.**, Pabo, C.O., Moreno, V., Kempf, A., Pyati, J., and Shankley, N.P. (2003) Cell lines for Drug Discovery: Elevating Target-protein Levels Using Engineered Transcription Factors. Journal of Biomolecular Screening. In Press.

Snowden, A.W., Zhang, L., Urnov, F., Dent, C., Jouvenot, Y., Zhong, X., Rebar, E.J., Jamieson, A.C., **Case, C.C.**, Pabo, C.O., Wolffe, A.P. and Gregory, P.D. (2003) Repression of Vascular Endothelial Growth Factor-A in Glioblastoma Cells Using Engineered Zinc-finger Transcription Factors. Cancer Res. In Press.

Tan, S., Guschin, D., Davlos, A., Lee, Y-L., Snowden, A.W., Jouvenot, Y., Zhang, S., Howes, K., McNamara, A.R., Lai, A., Ullman, C., Reynolds, L., Moore, M., Campos, B., Qi, H., Spratt, S.K., **Case, C.C.**, Pabo, C.O., Campisi, J., and Gregory, P.D. (2003) ZFP-targeted Gene Regulation: Genome-wide Single Gene Specificity. Proc. Natl. Acad. Sci. (USA) 100: 11997-12002.

Bartsevich, V.V., Miller, J.C., **Case, C.C.**, and Pabo, C. O. (2003). Engineered Zinc Finger Proteins for Controlling Stem Cell Fate. Stem Cells. In Press.

Case, C.C. (2003). Transcriptional Tools for Aging Research. Mechanisms of Aging and Development 124:103-108.

Snowden, A. W., Gregory, P.D., **Case, C.C.**, and Pabo, C.O. (2002). Gene-specific Targeting of H3K9 Methylation is Sufficient for Initiating Repression *in vivo*. Current Biology 12:2159-2166.

Liang, Y., Li, X-Y., Rebar, E.J., Li P-X., Zhou, Y., Chen, B., Wolffe, A.P. and **Case, C.C.** (2002). Identification and Characterization of a Cell-type Specific Enhancer that Regulates Transcriptional Activation of Vascular Endothelial Growth Factor A in Tumorigenic Glioblastoma Cell Lines. J. Biol. Chem. 277: 20087-20094.

Schaal, T.D., Holmes, M.C., Rebar, E.J., and **Case, C.C.** (2002). Novel Approaches to Controlling Transcription. Genetic Engineering, Principles and Methods vol. 24. 137-178. Jane Setlow ed.

Rebar, E.J., Huang, Y., Nath, A.K., Hickey, R., Meoli, D., Nath, S., Spratt, S.K., **Case, C.C.**, Wolffe, A.P., and Giordano, F.J. (2002). Induction of Angiogenesis in a Mouse Model using Engineered Transcription Factors. Nature Medicine 8: 1427-1432.

Publications (cont.)

Liu, Q., Xia, Z. and **Case, C.C.** (2001). Validated Zinc Finger Protein Designs for all 16 GNN Triplet DNA Targets. J. Biol. Chem. 277:3850-3856.

Liu, P-Q., Rebar, E., Zhang, L., Liu, Q., Jamieson, A., Liang, Y., Qi, H., Li, P-X., Chen, B., Mendel, M., Zhong, X., Lee, Y-L., Eisenberg, S., Spratt, S.K., **Case, C.C.**, Wolffe, A. (2001). Regulation of an Endogenous Locus Using a Panel of Designed Zinc Finger Proteins Targeted to Accessible Chromatin Regions: Activation of Vascular Endothelial Growth Factor A. J. Biol. Chem. 276:11323-11334.

Liu, P-Q., Rebar, E., Zhang, L., Liu, Q., Jamieson, A., Liang, Y., Qi, H., Li, P-X., Chen, B., Mendel, M., Zhong, X., Lee, Y-L., Eisenberg, S., Spratt, S.K., **Case, C.C.**, Wolffe, A. (2001). Regulation of the Endogenous VEGF-A Chromosomal Locus using Designed Zinc Finger Proteins. Biochemistry and Cell Biol. 79:377.

Zhang, L., Spratt, S.K., Liu, Q., Johnstone, B., Qi. H., Raschke, E., Jamieson, A., Rebar, E., Wolffe, A., **Case, C.C.** (2000). Synthetic Zinc Finger Transcription Factor Action at an Endogenous Chromosomal Site: Activation of the Human Erythropoietin Gene. J. Biol. Chem. 275:33850-33860.

Medina JC; Shan B; Beckmann H; Farrell RP; Clark DL; Learned RM; Roche D; Li A; Baichwal V; **Case, C.C.**; Baeuerle PA; Rosen T; Jaen JC. (1998). Novel Antineoplastic Agents With Efficacy Against Multidrug Resistant Tumor Cells. Bioorganic & Medicinal Chemistry Letters. 8:2653-6.

Case, C.C. and Rosen, T. (1994) 3-alkoxybenzo(b)thiophene-2-carboxamides as inhibitors of neutrophil-endothelial cell adhesion. Chemtracts Org. Chem. 7:329-332.

Case, C.C. and Dhundale, A.R. (1991) Gene regulation by antisense RNA and DNA: a meeting review. Antisense Res. And Dev. 1:207-217.

Case, C.C., Simons, E.L. and Simons, R.W. (1990) The IS10 transposase messenger RNA is destabilized during antisense RNA control. EMBO 8:4297-4306.

Case, C.C., Roels, S.M., Jensen, P.D., Lee, J., Kleckner, N. and Simons, R.W. (1989) The unusual stability of the IS10 antisense RNA is crucial for function and is determined by the structure of its stem domain. EMBO 8:4297-4306.

Case, C.C., Roels, S.M., Gonzalez, J.E., Simons, E.L. and Simons, R.W. (1988) Analysis of the promoters and transcripts involved in IS10 antisense RNA control. Gene 72: 219-236.

Case, C.C., Bukau, B., Granett, S., Villarejo, M.R. and Boos, W. (1986) Contrasting mechanisms of EnvZ control of the Mal and Pho regulons in Escherichia coli. J. Bacteriol. 166: 706-712.

Publications (cont.)

Villarejo M; **Case C.C.** (1984). *envZ* Mediates Transcriptional Control by Local Anesthetics but is not Required for Osmoregulation in *Escherichia coli*. Journal of Bacteriology 159:883-7.